March 12, 1950.

Dr. M. R. Zelle,
5624 Greentree Road,
Bethesda, Maryland.

Dear Max:

I've completed study of your 2/21 and 2/24 series, confirming the postcard note in fair detail. There were no genetic changes at all in the 2/21 series. In 2/24: A21 & A24 were indistinguishable (diploid), so your supposition on the basis of the "Harvard principle" (max. unhappiness) is unfounded. B28 and B30 are both segregants; B29 diploid -- I assume that B28 and 30 were actually sibs, since you boxed this clone. C was all diploid. D30 was a segregant (Lac-Mal-); I think D29 is a typical diploid, but am rechecking. F was all diploid, but may be peculiar in giving an unusually high proportion of Mal- segregants in every case. Other segregants are still just as mentioned in the postcard.

The effects of radiations on diploids have taken up most of my time since Christmas, with results much like those we discussed at the AAAS. UV seems to "haploidize", even with relatively small doses, before there is much killing. Treatment with higher UV doses, followed by photoreactivation is equivalent (qualitatively) to a correspondingly reduced dose of UV, dark. Novick finds, however, that the parents, as well as the segregants, of H226 are of two types in their response to photoreactivation. One gives linear or constant dose-reduction for all UV doses; the other gives very little dose reduction for low UV, with an increasing photoreactivable fraction of the dose, as the dose is increased. Roughly, the diploid seems to follow the former behavior, suggesting that this may be controlled by a dominant genetic factor. The initial killing is easily accounted for by the haploidization (which I assume reflects the destruction of single chromosomes or nuclei), but I have been surprised to find that the fraction of diploids among the survivors does not continue to decrease with increasing doses, but levels off, or else decreases very much less abruptly when this fraction is about 15-20%. This may reflect either a superimposed, differential killing mechanism, or a heterogenity in the cells (possibly a fraction with many diploid nuclei), probably the latter. If balanced lethals occur at all, they are quite rare. By screening on a large scale (i.e., allowing segregation to occur en masse), and selecting for the residual diploids), I have been able to pick up a few possible stabilized diploids, but even most of these are uncertain. The haploidization mechanism probably preponderates very heavily.

Some earlier experiments I did seemed to rule outppartial haploidizations, i.e., chromosome deletions of more limited extent, but when I used selective methods, I found quite a number of cultures after UV which still segregated ac v, Mal- resp for ~~maltose~~, but were ~~Lac~~ However, when the same procedure was applied to control cultures which had been allowed to grow on complete medium, the same sort of thing was found, albeit with a low, unfortunately not estimable, frequency. Also, one Mal- Lac y has been found. The exceptional clone of the previous set of isolations may be a short-lived representation of this sort of separate segregation for Mal and Lac, and I cannot avoid the feeling that we are hot on the trail of some useful answers. The partial segregants are being tested to see whether they are, e.g., ~~Lac~~ Lac- Mal-/Lac+ Mal- or Laq- Mal-/ Lac+ df., which is what the typical diploids seem to be.

Another consideration that comes from the radiation experiments, that also helps to justify the hope that they will give some information on the structure of the diploid. The UV-induced haploids are indistinguishable, so far, from the spontaneous segregants. Although not ruled out, induced meiosis is, I think, highly improbable. On the other hand, it seems to me possible that many, but not all, of the spontaneous segregants may be the spontaneous loss counterparts of the UV induced haploids. That is, the spontaneous haploids may not arise by meiosis, but byspontaneous breakage or loss of one chromosome. This would account very neatly for the absence of reciprocal segregants in most instances, even in H226 and H206, as well as for the greatly reduced amount of crossing-over which characterizes the segregants of persistent diploids as compared with selected prototrophs. It may be possible to evaluate this by study of the partial segregants, in particular if they can be found in your pedigrees.

In addition to UV, the genetic effects on diploids of X-rays and of a variety of chemicals have been studied. X-rays give qualitatively the same effects, although they give a much more nearly exponential killing curve. Also, a variety of chemicals have the same haploidizing effects in association with killing. These include: Nitrogen mustard; acetic anhydride; formaldehyde; dimethyl sulfate, all of which are effective alkylating agents -- which should suggest a mechanism of radiation action: immediate or ultimate production of free radicals? Other agents kill without associated haploidization: heat, basic dyes; iodoacetamide; ninhydrin. Some other compounds are being tested now to test the generalization of the radiomimetic effects of alkylating agents, which should not be expected to hold up indefinitely.

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urethan,

Now to some more prosaic matters. I've just sent off a third return shipment of vials -- have you received the others, which were mentioned in my postcards? I can also get hold of the caps separately; wire or write if you need anything. How about my sending you the vials with nutrient broth (or whatever you use) and already sterilized? Or do you work from other glassware. Is there anything else along this line we can do-- just mention it. Also, do you want me to continue addressing letters and packages to your home?

I had an interesting, perhaps somewhat depressing, visit to Oak Ridge. There is no serious question of my leaving Madison, and certainly not to a secret installation to do nonsecret work. Can we expect a visit from you sometime this Spring?

Sincerely,

Joshua Lederberg